REMARKS

Reconsideration of the allowability of the present application is requested respectfully.

Status of the Claims

Claims 1 to 11 and 21 to 30 are pending. No claims have been allowed. No claims have been amended. No claims have been cancelled. Claim 31 has been added. Accordingly, Claims 1 to 11 and 21 to 31 are presented for examination.

Claim 31 recites a method for preserving bone marrow stromal cells (BMSCs). Support for this claim may be found from page 11, line 20, to page 12, line 2, and on page 18, lines 1 to 20 of the application.

In response to the Examiner's final Action dated September 19, 2002, applicants traverse respectfully the Examiner's rejection of Claims 1 to 11 and 21 to 30.

Summary of the Rejections

The Examiner has rejected Claims 1 to 5, 7 to 10, 21, 22, 24 to 26, 29 and 30 under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 5,399,346 ("Anderson et al.") taken with European Patent Application No. 0381490 ("Greenberger et al.") and Boswell et al. Exp. Hematol. 11:315-323 (1983).

The Examiner has rejected Claims 1 to 10, 21 to 26, 29 and 30 under 35 U.S.C. §103(a) as being unpatentable over Anderson et al., Greenberger et al., and Boswell et al., and in further view of Lozier et al. Hum. Gene Ther. 5:313-322 (1994).

The Examiner has rejected additionally Claims 1 to 5, 7 to 11, 21, 22, and 24 to 30 under 35 U.S.C. §103(a) as being unpatentable over Anderson et al., Greenberger et al., and Boswell et al., and further in view of Lobb et al. BBRC 178:1498-1504 (1991).

Each of these rejections is discussed below in the order in which they were presented.

The 35 U.S.C §103(a) Rejections

The Examiner's §103(a) rejection of Claims 1 to 5, 7 to 10, 21, 22, 24 to 26, 29 and 30 as being unpatentable over Anderson et al., taken with Greenberger et al. and Boswell et al. is traversed respectfully.

Anderson et al. discloses transfection of tumor-infiltrating lymphocytes (TILs) and the cryopreservation of transfected TILs. Greenberger et al. discloses using bone marrow stromal cells (BMSCs) for gene therapy, but does not teach cryopreservation of BMSCs. Boswell et al. discloses cryopreservation of untransfected bone marrow cells.

In the arguments presented below, applicants will demonstrate there is no suggestion or motivation to combine the cited publications. In particular, applicants will demonstrate that one of skill in the art would not be motivated to combine the cited publications based on contemporaneous scientific literature

None of the cited publications contains any suggestion or motivation to modify or combine the publication teachings so as to render the claimed invention obvious. One of skill in the art would not combine a publication on TILs (Anderson et al.) with a publication on BMSCs (Greenberger et al. and Boswell et al.). Guidance on the requirements for combining publications in an obviousness rejection is provided by the MPEP §2143. "The factual inquiry whether to combine references must be thorough and searching. It must be based on objective evidence of record." *In re Sang-Su Lee*, 277 F. 3d 1338, 1343, (C.A.Fed. 2002)

emphasis added; see MPEP (8th Ed. Rev. 1 Feb. 2003) §2143.01. Also, "the Board must identify specifically the principle, known to one of ordinary skill, that suggests the claimed combination. In other words, the Board must explain the reasons one of ordinary skill in the art would have been motivated to select the references and to combine them to render the claimed invention obvious." *In re Rouffet,* 149 F.3d 1350, 1359, (Fed.Cir.1998). The Examiner has provided no reason as to why one would select a TIL publication and combine it with a BMSC publication.

The Examiner has attempted to provide reasons why one of skill in the art would be motivated to make the claimed invention. But such motivation is not what is required by MPEP §2143, namely, a motivation to combine reference teachings. "The PTO's theory that one might have been motivated to try to do what [the inventors] in fact accomplished amounts to speculation and an impermissible hindsight reconstruction of the claimed invention." *In re Deuel*, 51 F.3d 1552, 1558, (C.A.Fed. 1995). What is missing from the Examiner's prima facie case of obviousness is evidence why one of skill in the art would be motivated to particularly choose Anderson et al. and combine it with Greenberger et al. and Boswell et al. What is the motivation for a skilled artisan, knowing that TILs and BMSCs respond differently to cryopreservation, to choose the teachings of a TIL reference in order to successfully cryopreserve BMSCs? Conclusory statements regarding such motivation, such as "because the claimed invention is beneficial", do not satisfy the requirements of the USPTO for a §103 rejection.

Applicants have provided substantive reasons (reiterated below) for why one would not combine a TIL publication with a BMSC publication. In contrast, the Examiner has not provided a proper, rule-based basis to combine the publications. As explained above and in the cited cases, the mere recognition that cryopreserved transfected BMSCs are desirable

does not satisfy the requirement that the Examiner provide objective evidence as to why a skilled artisan would combine a TIL publication with a BMSC publication.

Applicants have explained that TILs and BMSCs are fundamentally different types of cells that react differently to cryopreservation. To support this position, Applicants submitted two exhibits with the Reply dated August 23, 2002: a publication by Zaheer et al. ("Differential sensitivity to cryopreservation of clonogenic progenitor cells and stromal precursors from leukemic and normal bone marrow," Stem Cells 12:180-186 (1994)) and an information sheet from Mediatech, Inc.

Zaheer et al. was published in 1994 and thus is an example of the state of the cryopreservation art at the time of the earliest priority date for the present application (December 29, 1995). The Mediatech document was copyrighted in 2001 and made available on the internet at an unknown date.

To help clarify Applicants' position the experimental findings disclosed in Zaheer et al. are summarized below. However, before discussing Zaheer et al. applicants believe it would be helpful to provide some background information on bone marrow cells and stem cells

Bone marrow comprises many different cell types. Among these cell types are stem cells. Stem cells can differentiate into many other cell types. Before stem cells become fully differentiated, they first go through an intermediate or progenitor phase wherein the cells are committed to a specific lineage, but are technically not fully differentiated. These progenitors later become fully differentiated cells (e.g., a lymphocyte or TIL). One common characteristic shared by stem cells, progenitor cells, and the fully differentiated cells is that they are all nonadherent cell types.

Stromal cells are also found in bone marrow. In contrast to nonadherent cell types, stromal cells grown *in vitro* form a layer that adheres to a tissue culture dish. Thus, they are referred to as "adherent" cells. It is undisputed that one of skill in the art would recognize that a TIL is similar to a non-adherent progenitor cell and that, in contrast, a BMSC is an adherent stromal cell.

Zaheer et al. cryopreserved bone marrow cells (a mixture of stromal cells and progenitor cells) and then thawed the cells. These thawed cells were compared to bone marrow cells that had never been frozen. In this regard, the cryopreserved progenitor cells acted very similarly to non-cryopreserved progenitor cells. The cryopreserved progenitor cells were able to proliferate and differentiate just as well as non-cryopreserved progenitor cells.

A very different result was seen for the stromal cells. The stromal cells recovered after cryopreservation could not form a stromal layer (see paragraph bridging pages 182 and 183 of Zaheer et al.). Also, and more critically, cryopreserved stromal cells could not support proliferation and differentiation of progenitor cells (page 182, column 2, lines 13 to 20) as measured by using long-term bone marrow culture (LTBMC). LTBMC is an assay comprising growing progenitor cells in the presence of a stromal layer and then determining if proliferation and/or differentiation of progenitors occurs. Thus, when cryopreserved progenitor cells were grown on a non-cryopreserved, pre-formed stromal layer, proliferation and differentiation did occur (page 185, column 2, lines 11 to 15). When cryopreserved progenitor cells were grown on a cryopreserved stromal layer, proliferation and differentiation did not occur (page 182, column 2, lines 13 to 20).

The conclusion of Zaheer et al. is that cryopreservation did not affect the progenitor cells but did have an effect on the stromal cells. Thus, adherent stromal (including BMSCs)

cells react differently to cryopreservation than do nonadherent progenitor cells (similar to TILs).

Applicants submit that in responding to applicants' arguments based upon Zaheer et al., the Examiner mischaracterized Zaheer et al. On page 3 of the present Action, the Examiner states that "the teachings of Zaheer et al. do not negate the feasibility of cryopreserving BMSCs, rather, they teach even though the stromal cells are more sensitive to the process of freezing and thawing, they are still alive and growing". This statement is both incorrect and incomplete. On page 183, column 1, lines 1 to 3, Zaheer et al. states: "frozen BMMC [bone marrow mononuclear cells] from either leukemic or normal bone marrow failed to form a confluent stroma even after four to five weeks". "Failure to form a confluent stroma" indicates that while this stromal layer is "alive", it certainly is not "growing". Furthermore, the Examiner has not considered that, as a consequence of cryopreservation, this thawed stromal layer no longer can support progenitor growth and differentiation. Thus, this stromal layer is neither growing nor functioning.

Applicants submitted the Zaheer et al. publication to demonstrate the thinking in the art about the time of the present invention and to further demonstrate why the skilled artisan would not combine Anderson et al. with either Greenberger et al. or Boswell et al. Clearly, cryopreservation has affected the ability of the stromal cells to grow and express genes necessary to support progenitor growth and differentiation. In contrast, cryopreservation does not affect the ability of progenitor cells (which are precursors to TILs) to grow and differentiate.

Applicants submit that, in view of Zaheer et al., one of skill in the art would not look to a method of cryopreserving TILs in order to cryopreserve transfected BMSCs. Thus, there is no suggestion/motivation to combine the cited publications as the Examiner has done.

Applicants have previously provided the information sheet from Mediatech, Inc. in order to emphasize that cryopreservation solutions for adherent and nonadherent cells differ. Because these cryopreservation solutions differ, one of skill in the art would not look to a nonadherent cell cryopreservation method, when cryopreserving adherent cells. Anderson et al. discloses transfection of tumor-infiltrating lymphocytes (TILs) and the cryopreservation of transfected TILs. TILs are nonadherent cells. The present invention is directed to bone marrow stromal cells (BMSCs). BMSCs are adherent cells. Thus, one of skill in the art would not look to Anderson et al. when cryopreserving BMSCs. As Zaheer et al. demonstrates, when BMSCs are not cryopreserved properly, they will not function properly. Any random cell cryopreservation method that gives a few viable BMSCs will not suffice.

On page 4 of the present Action, the Examiner has characterized the cryopreservation method of the present application as "standard". In support of this characterization, the Examiner cites Boswell et al. and Zaheer et al. as having the same freezing solution (10% DMSO and 90% medium). This is not correct. The freezing solutions of Boswell et al., Zaheer et al., and the present invention are all different. Boswell et al. discloses a freezing solution of 10% DMSO, 18% heat inactivated fetal calf serum, and 72% McCoy's 5A medium (page 317, column 1, second full paragraph). Zaheer et al. discloses a freezing solution of 10% DMSO, 45% fetal calf serum, and 45% Iscove's modified Dulbecco's medium (page 181, column 2, first full paragraph). The present application discloses a freezing solution of 10% DMSO, 1-50% fetal bovine (i.e., calf) serum, and 89-40% Dulbecco's modified Eagle's medium (page 11, lines 20 to 29 and page 18, line 1 to page 19, line 2). It should be noted that for all the reliance put on the Anderson et al. publication, this publication does not disclose any freezing solution or media at all. Since the two publications

of record in this case utilize different freezing solutions from the present application, a characterization of the present cryopreservation solution as "standard" has no basis.

Furthermore, the freezing methods of Boswell et al. and Zaheer et al. differ from those of the present invention. In Boswell et al., the cells were slowly cooled (1°C/min to -30°C; 10°/min between -31°C and -90°C) prior to liquid nitrogen storage. In Zaheer et al., the cells were placed in dry ice for four hours prior to liquid nitrogen storage. In the present application, BMSCs were placed at -80°C for 24 hours prior to liquid nitrogen storage.

Thus, the different cryopreservation solution and method of the present application distinguishes the invention over the combination of Anderson et al., taken with Greenberger et al. and Boswell et al. The freezing solution and methods of the present application (which is used for BMSCs) are different than the freezing solution and methods of Boswell et al. (which is used for total bone marrow cell preparations).

The Examiner has rejected Claims 1 to 10, 21 to 26, 29 and 30 under 35 U.S.C. §103(a) as being unpatentable over Anderson et al. in view of Greenberger et al., and Boswell et al. and further in view of Lozier et al. (*Hum. Gene Ther.*).

Applicants respectfully traverse the rejection.

Lozier et al. has been applied for the teaching of a canine model. As discussed in previous replies, the teaching of a canine model provides no basis to overcome the deficiencies of Anderson et al., Greenberger et al., and Boswell et al. as noted above. Since Lozier et al. does not provide any information that overcomes the deficiencies in the other cited publications, applicants request respectfully withdrawal of the obviousness rejection of Claims 1 to 10, 21 to 26, 29 and 30 which additionally relies on Lozier et al.

Claims 1 to 5, 7 to 11, 21, 22, and 24 to 30 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Anderson et al. in view of Greenberger et al., and Boswell et al., and further in view of Lobb et al. (*Biochem. Biophy. Res. Com.*).

The Examiner's §103(a) rejection of Claims 1 to 5, 7 to 11, 21, 22, and 24 to 30 is traversed.

The Examiner has asserted on page 8 of the Action that "Lobb et al. not only contemplated that VCAM-1 is a surface molecule and could be used for leukocyte targeting, they in fact have transfected cells with the full-length VCAM previously". Lobb et al. discloses the transfection and expression of secreted recombinant soluble VCAM-1 (rsV-CAM-1) in CHO cells. rsVCAM-1 lacks a transmembrane region (see Figure 1 of Lobb et al.) and thus is not a cell surface molecule. The Examiner has cited the last paragraph of page 1503 of Lobb et al. to support this rejection. This paragraph states (emphasis added by Examiner):

> In summary, we have generated milligram quantities of a soluble monomeric form of human VCAM1. rsVCAM1 can serve as a functional adhesion protein demonstrating the same specificity as VCAM1 expressed at the surface of HUVECs, and should prove useful for the evaluation of the effects of VCAM1/VLA4 cognate recognition on leukocyte function.

Applicants submit that there is nothing in this passage that suggests that Lobb et al. transfected any cell, especially HUVECs, with full-length VCAM-1. HUVECs already express VCAM-1 endogenously. The first sentence on page 1498 (following the abstract) of Lobb et al. states: "VCAM-1 is a member of the immunoglobulin (Ig) superfamily which is expressed on human umbilical vein endothelial cells (HUVECs)". Thus, Applicants reiterate that Lobb et al. does not disclose transfection and expression of any cell surface molecules and specifically does not disclose transfection and expression of VCAM-1. Thus, the Examiner's assertion that Lobb et al. can be applied for the teaching of expressing cell

surface molecules is without basis. In addition, Lobb et al. does not provide any information that overcomes the deficiencies in the other publications. Accordingly, applicants respectfully request withdrawal of the §103(a) rejection of Claims 1 to 5, 7 to 11, 21, 22, and 24 to 30.

Enclosed herewith is a Petition for a three-month extension of time to respond to the Examiner's Action. Also enclosed in duplicate is a second Notice of Appeal, which supercedes the Notice of Appeal filed August 23, 2002. In view of the Examiner's vacation of finality of the Office Action dated April 23, 2002, Applicants request that, if possible, the fee for the Notice of Appeal filed August 23, 2002 be refunded and applied to the Notice of Appeal filed herewith. The Commissioner is hereby authorized to credit the overpayment associated with this communication to Deposit Account No. 19-5425. The Commissioner is also hereby authorized to charge any additional fees associated with this communication to Deposit Account No. 19-5425.

Respectfully submitted,

Patrick J. Kelly, Ph.D.
Registration No. 34,638

SYNNESTVEDT & LECHNER LLP
Suite 2600 Aramark Tower
1101 Market Street
Philadelphia, PA 19107
(215) 923-4466